Crystallization of Haloalkane Dehalogenase from Xanthobacter autotrophicus GJ10

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Haloalkane dehalogenases are enzymes that release chloride or bromide from *n*-halogenated alkanes. X-ray quality crystals of haloalkane dehalogenase from the 1,2-dichloroethane-degrading bacterium *Xanthobacter autotrophicus* GJ10 have been grown at room temperature from 64% saturated ammonium sulfate solutions (pH 6·2 to 6·4). The crystals diffract in the X-ray beam to at least 2·4 Å resolution (1 Å = 0·1 nm). Their space group is $P2_12_12$, with cell dimensions $a=94{\cdot}1$ Å, $b=72{\cdot}8$ Å, $c=41{\cdot}4$ Å and $\alpha=\beta=\gamma=90°$. There is one monomer (molecular weight 36,000) per asymmetric unit.

The nitrogen-fixing hydrogen bacterium *Xanthobacter autotrophicus* GJ10 is capable of growth with 1,2-dichloroethane or 2-chloroethanol as a sole source of carbon and energy (Janssen *et al.*, 1984, 1985). A few other haloalkanes and halocarboxylic acids also support growth, such as bromoethane, 1-chlorobutane or dichloroacetic acid. In order to utilize these compounds, the organism produces enzymes that release chloride or bromide from these halogenated chemicals. Two different enzymes have been found so far: one enzyme is specific for halogenated alkanes, whereas the other acts on halogenated carboxylic acids. Both dehalogenases in strain GJ10 are constitutively produced and no induction of these enzymes by haloalkanes that serve as carbon source has been observed. The two dehalogenases are clearly distinct in substrate specificity, heat stability and pH optimum, and they do not copurify upon ammonium sulfate fractionation or ion-exchange chromatography (Janssen *et al.*, 1985).

To investigate the action of these enzymes in more detail, haloalkane dehalogenase has been purified to homogeneity (Keunig *et al.*, 1985). This enzyme consists of a single polypeptide chain with a molecular weight of 36,000. It has an isoelectric point of 5·4 and maximum activity was found at pH 8·2. The amino acid composition suggests the presence of one cysteine residue. Thiol reagents such as $HgCl_2$, iodoacetamide, *p*-chloromercuribenzoate and *N*-ethylmaleimide readily inhibit the enzyme, indicating that this cysteine is most probably involved in catalysis and is located in or near the active site. The enzyme converts haloalkanes to their corresponding alcohols without a requirement for oxygen or cofactors, suggesting a nucleophilic displacement with water as the mechanism for halide release. A number of different halogenated alkanes are hydrolyzed by the enzyme, among which are the environmentally important chlorinated hydrocarbons methylchloride, ethylchloride and 1,2-dichloroethane (McConnell *et al.*, 1975). These compounds are considered priority pollutants by the U.S.A. Environmental Protection Agency (Patterson & Kodukala, 1981). Carbon–chlorine, carbon–bromine and carbon–iodine bonds of monohaloalkanes are hydrolyzed by the enzyme, but the enzyme has no activity towards chlorinated aromatic hydrocarbons or chlorinated ethylenes. A plausible mechanism for enzymatic hydrolytic dehalogenation *via* a thiol group has been given by Goldman (1965). He hypothesized that the reaction proceeds *via* a covalent intermediate, in which a sulfhydryl group in the enzyme displaces the halogen of the haloalkane to form a thioether bond with the substrate. This thioether is then hydrolyzed by a hydroxyl group to give an alcohol.

To obtain more insight into the mechanism of the catalytic reaction and to learn which factors determine the substrate specificity, we embarked on a crystallographic investigation of dehalogenases. This paper reports on the crystallization of haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10.

Haloalkane dehalogenase was prepared in large quantities as described (Keuning *et al.*, 1985). The protein, which was dissolved in buffer (pH 7·5: 50 mM-Tris-H_2SO_4, 100 mM-$(NH_4)_2SO_4$, 1 mM-EDTA, 1 mM-β-mercaptoethanol) was concentrated to 8·1 mg/ml (assuming $A_{280}=1{\cdot}0$ unit at a concentration of 1·2 mg protein/ml). Subsequently the protein was dialyzed twice for 24 hours against 0·1 M-bis-Tris-H_2SO_4 buffer (bis(2-hydroxyethyl)-imino-tris(hydroxymethyl)methane; pH 6·4). Crystals were grown at room temperature by vapor diffusion in hanging drops (5 μl of protein solution and 5 μl of precipitant) suspended over a 1 ml reservoir of the precipitant. As a precipitant we used 50 to 70% saturated ammonium sulfate in the above mentioned bis-Tris-H_2SO_4 buffer (pH 5·4 to 7·2). Crystals appeared at ammonium sulfate concentrations of 60% and higher, at all pH values tested between 5·4 and 7·2. The largest crystals grew at 64% saturation, and pH 6·2 to 6·4. The colorless crystals are lozenge-shaped, with average diameters 0·4 and 0·5 mm, and thickness 0·15 mm. Because of slight pH-dependent variations in cell dimensions, the crystals were soaked for 16 to 24 hours in the mother liquor (pH 6·2), before being mounted in capillaries. The crystals belong to the

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orthorhombic space group $P2_12_12$, with unit cell dimensions $a = 94{\cdot}1$ Å, $b = 72{\cdot}8$ Å, $c = 41{\cdot}4$ Å and $\alpha = \beta = \gamma = 90°$, which corresponds to a unit cell volume of 283,610 Å^3 (1 Å = 0·1 nm). The volume per unit mass, V_M (Matthews, 1968), is 2·0 Å^3/dalton, assuming one molecule of dehalogenase per asymmetric unit. This is within the range 1·6 to 3·6 found to be typical for protein crystals. The crystals diffract to at least 2·4 Å resolution, and are stable for over ten days in the X-ray beam from a conventional generator operated at 40 kV and 40 mA.

A native structure dataset has been collected by oscillation photography to 2·4 Å resolution from one crystal, with two other crystals used for collecting the cusp data, at the EMBL outstation at the DESY synchrotron in Hamburg. A search for heavy-atom derivatives is in progress.

The availability of an X-ray structure is a prerequisite for detailed mechanistic studies of the haloalkane dehalogenase. Long-term goals to modify substrate specificity by site-directed mutagenesis so that the enzyme can have specific industrial applications is also clearly dependent upon knowledge of the three-dimensional structure, This report of the crystallization of the enzyme is a first step towards these goals.

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